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The legacy of pesticide pollution: An overlooked factor in current risk assessments of freshwater systems

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ABSTRACT

We revealed a history of legacy pesticides in water and sediment samples from 19 small streams across an agricultural landscape. Dominant legacy compounds included organochlorine pesticides, such as DDT and lindane, the organophosphate chlorpyrifos and triazine herbicides such as terbutylazine and simazine which have long been banned in the EU. The highest concentrations of legacy pesticides were found in streams draining catchments with a large proportion of arable farmland suggesting that they originated from past agricultural applications. The sum of toxic units ($SumTU_{D.magna}$) based on storm water samples from agriculturally impacted streams was significantly higher when legacy pesticides were included compared to when they were omitted. Legacy pesticides did not significantly change the predicted toxicity of water samples to algae or fish. However, pesticide concentrations in bed sediment and suspended sediment samples exceeded safety thresholds in 50 % of the samples and the average contribution of legacy pesticides to the $SumTU_{C.riparius}$ was > 90%. Our results suggest that legacy pesticides can be highly significant contributors to the current toxic exposure of stream biota, especially macroinvertebrate communities, and that those communities were primarily exposed to legacy pesticides via the sediment. Additionally, our results suggest that neglecting legacy pesticides in the risk assessment of pesticides in streams may severely underestimate the risk of ecological effects.

KEYWORDS: Legacy pesticides, Environmental Risk Assessment, Mixture Toxicity, Pesticide Monitoring, Streams

INTRODUCTION

Publication frequency of articles characterising the contamination dynamics of freshwater systems in space and time has increased over the past decade in recognition of the need to increase realism of current exposure and risk assessments to support an informed management of these systems. Pesticides in particular have received increasing attention given their suggested important role in the global loss of freshwater biodiversity and ecosystem functioning (Beketov et al. 2013; Malaj et al. 2014; Rasmussen et al. 2012; Schäfer et al. 2012). In this article, we subdivide pesticides into those still registered for agricultural use in the European Union and in Denmark (referred to as contemporary pesticides) and those that have been discontinued or banned for usage in conventional agriculture (referred to as legacy pesticides).

Pesticides applied to agricultural fields may reach surface water through a series of different pathways with surface runoff and tile drains being widely accepted as the most important routes for contemporary pesticides (Schulz 2004). These transport routes are primarily initiated during heavy precipitation events and lead to transient peak concentrations often exceeding current ecological quality criteria (Bundschuh et al. 2014; Liess and von der Ohe 2005; Schulz 2004). In contrast, legacy pesticides may enter surface water continuously via groundwater inflow (Barth et al. 2007; Gilliom 2007; McKnight et al. 2015), atmospheric deposition (Konstantinou et al. 2006; Weber et al. 2010) or through continuous leaching from agricultural soils and landfills (Aliyeva et al. 2013). Consequently, legacy pesticides may generate a relatively constant exposure regime in surface waters. The yearly flux of legacy pesticides to freshwater ecosystems may comprise up to several percent of the historical yearly applied amounts in a catchment (Barth et al. 2007). Importantly, pesticides and their residues may persist and even accumulate in sediments of freshwater ecosystems (Dai et al. 2014; Kuivila et al. 2012; Nowell et al. 2013).

Factors controlling the fate of a pesticide in agricultural landscapes include a variety of chemical and environmental properties of the pesticide (e.g. degradation rate, adsorption to organic carbon and water solubility), climatic factors (e.g. temperature and precipitation), soil characteristics, topography and agricultural practices (Leonard 1990; Wauchope 1978). More than 20,000 pesticide products have entered the market since registration became legislatively required in 1947, and it is therefore not surprising that the combined effect of multiple factors influencing the environmental transport and fate of each pesticide generates highly complex exposure profiles of pesticide mixtures in time and space (Konstantinou et al. 2006; Wauchope 1978). However, pesticides that are currently applied in the highest quantities are also those that occur most often in surface waters with the more water soluble and persistent compounds reaching the highest concentrations (Bundschuh et al. 2014; Kreuger and Tornqvist 1998; Li et al. 2013; Moschet et al. 2014). Therefore, current pesticide usage is often used to guide the prioritisation of active ingredients included in monitoring programmes and research activities. Moschet et al. (2014) showed that a stringent focus on EU priority pollutants or a subset of the active ingredients applied in the highest quantities on the national level may seriously underestimate predicted toxic pressures in streams. Whereas Moschet et al. (2014) aimed to document that an extensive pesticide screening (249 active ingredients) translates into significantly higher predicted mixture toxicities compared to screenings restricted to fewer pesticides (≤ 36), the authors did not distinguish between the toxic contribution of contemporary and legacy pesticides. Based on water samples mainly analysed for herbicides and four sediment samples mainly analysed for insecticides, McKnight et al. (2015) suggested that legacy pesticides could still be prominent players driving observed impairments of freshwater invertebrates, and the authors urged for more extensive studies that allow for quantifying the predicted toxicological potency of legacy pesticides in comparison to current use pesticides. To our

knowledge, such an extensive study of the potential toxicity of legacy pesticides to aquatic biota relative to that of contemporary pesticides is still lacking despite a substantial body of literature addressing the occurrence, concentrations and predicted toxicities of selected legacy pesticides (Aliyeva et al., 2013; Gilliom, 2007; McKnight et al., 2015; Weber et al., 2010). The novelty element is therefore to quantify the possible toxicity of legacy pesticides as an integral part of current risk assessment. Such an integration has a number of potentially vital implications for the usability of risk assessment, including that i) contemporary regulatory actions are only targeting substances that are still in use; ii) it gives an increased explanatory power in river quality assessment by quantifying the impact of current unknowns, which will additionally reduce the potential underestimation of the role of pesticides as stressors in stream ecosystems, which is currently most likely the case (Beketov et al., 2013; Malaj et al., 2014), and iii) it provides highly needed insight into pesticide exposure profiles in time and space that may be used as improved benchmarks for the interpretations of ecological response parameters.

This article aims to compare the toxicity of legacy pesticides and their metabolites to those of contemporary pesticides in 19 Danish 1st and 2nd order streams situated in agricultural landscape covering a range of agricultural intensity, local climate and soil types. Water samples were collected during base flow and peak flow for pesticide analyses, and bulk sediment and suspended sediment were sampled to optimize detections of pesticides with low water solubility. In more detail, our objectives were to: i) characterize the occurrence of legacy pesticides in Danish headwater streams, ii) estimate the predicted toxicity of legacy pesticides and their residues using the Toxic Units (TU) approach, iii) evaluate the relative contribution of legacy pesticides and their residues to the summed TU of contemporary pesticides, and iv) evaluate which legacy pesticides are of highest ecotoxicological concern.

118

119 METHODS

120 *Study streams*

121 Nineteen Danish 1st and 2nd order streams (Fig. S1) were sampled for pesticide occurrences. Nine
122 streams with < 50% agricultural land-use in a two-sided buffer extending 2,000 m upstream of the
123 sampling site were selected in addition to 10 streams with expected high impact of pesticides
124 (conventional agriculture > 60% in the two-sided 100 m buffer). Furthermore, all study sites
125 complied with the following selection criteria: i) forest should occupy < 50% of a two-sided 50m
126 buffer extending from the study site and 2,000 m upstream, ii) proportional coverage of silt and
127 mud in stream substrates (indicative of drainage ditches) should be < 50%, and iii) no influence
128 from waste water treatment plants, but scattered settlements may influence the chemical water
129 quality. Detailed information on the study streams and catchments is provided in Table S1). In this
130 article, we refer to the nine streams with expected low agricultural impact as controls and the ten
131 streams with expected high agricultural impact as agricultural streams. All catchments are
132 characterised by loam or sandy loam, low elevation and precipitation ranges from ca. 800-850 mm
133 year^{-1} for central Jutland and on Funen and 700-750 mm year^{-1} on Zealand.

134

135 Base flow discharge was calculated as the product of the mean stream width, mean depth and mean
136 water velocity, based on measurements at ten transects along a 100 m stream reach extending
137 upstream from the sampling point (depth and velocity measured at 0, 25, 50 and 75% of the width
138 of each transect). Moreover, yearly mean discharge was estimated as the product of yearly mean
139 discharge coefficients (L s^{-1} km^{-2}), calculated for national hydrological monitoring stream sites
140 geographically/geologically selected as representative for the study streams, and catchment area for
141 the study streams (km^2). In a few cases national monitoring sites could not be regarded as truly

representative, and yearly mean discharge was designated as > base flow (Table S1). The proportion of conventional agriculture was quantified for the catchments of each study stream and for a two-sided 100 m buffer extending 2,000 m upstream of the sampling site were quantified in ArcGis 10.1 for windows.

Pesticide sampling

Sampling was conducted during May – August in 2012 coinciding with the main pesticide application season in this part of Europe. Dissolved phase pesticides were sampled with: i) manual grab samples in August during low flow conditions to optimize detections of pesticides originating from groundwater inflow (one sample per stream) and ii) event-triggered water samplers designed to capture water during storm flow (Liess et al. 1999). Manual collection of water samples during low-flow conditions were consistently preceded by one week without precipitation. Event-triggered water samplers were checked every week during May, June and July and collected if full, resulting in 64 storm flow water samples. The event-triggered water samplers strategically collect water representing a temporal point measurement during the first hours of a heavy rain incident (Liess et al. 1999).

Sediment associated pesticides were sampled with two different methods. Bed sediment was collected (top 1 cm) in depositional areas using Kajak corers (8 cm in diameter). Each bed sediment sample was comprised of 20-30 subsamples to obtain samples representative for the stream reach. Bed sediment was collected in all streams in mid-August reflecting newly deposited material during the summer period. Suspended sediment was additionally collected since the mobile sediment fraction may provide a stronger estimate for worst case scenarios (Liess et al. 1996). The Suspended Particle Samplers (SPS) used in this study are described in detail elsewhere (Laubel et al. 2001).

166

167 *Chemical analyses*

168 Water samples were screened for 70 pesticides and metabolites comprising 42 contemporary
169 pesticides, 26 legacy pesticides and 2 metabolites (Table S2). The 68 active ingredients included 35
170 herbicides, 16 fungicides and 17 insecticides. Bed sediment and suspended sediment samples were
171 screened for 38 pesticides and residues comprising 16 contemporary pesticides, 18 legacy pesticides
172 and 4 metabolites (Table S3). The 34 active ingredients included in the screening included 12
173 herbicides, 5 fungicides and 17 insecticides.

174

175 Analysis of water samples for the non-polar compounds was done by liquid/liquid extraction
176 followed by gas chromatography mass spectrometry (GC-MS). For the polar and semi-polar
177 compounds online solid-phase extraction followed by liquid chromatography tandem mass
178 spectrometry (LC-MS/MS) was performed as described by Jansson & Kreuger (2010).
179 Wet sediment sample (20 g) was mixed with a drying agent (10 g). A sub-sample of the mixture (9
180 g, corresponding to 6 g sediment) was placed in pre-cleaned (400 °C) glass fibre cartridges and
181 extracted together with the internal standards ethion and terbuthylazin-D5 by a Soxtec Avanti 2050
182 Auto System using dichloromethane and acetone (1:1). The extract was evaporated and diluted in
183 cyclohexane and dichloromethane (1:1) before purification by Gel Permeation Chromatography
184 (GPC), followed by evaporation and dilution in cyclohexane and acetone (9:1). The volume was
185 adjusted to 1 ml. The extract was injected on two separate GC-MS systems, one in negative
186 chemical ionization (NCI) mode (Agilent Technologies GC 7890, MS 5975C) and one in electron
187 impact (EI) mode (Agilent Technologies GC 6890, MS 5973), quantifying against an external
188 standard calibration. In order to enhance the sensitivity of the DDTs, a part of the initial extract was
189 purified with sulphuric acid and with the internal standards added once again before injection. The

190 standards used were obtained from Dr Ehrenstorfer GmbH. Dry-weight measurements of sediment
191 were performed in a dry oven (105 °C) during ca. 16 hours, with analytical results presented as µg
192 per kg of dry weight.
193
194 Values between the limit of detection (LOD) and the limit of quantification (LOQ) were given as trace
195 concentrations. At this level, the uncertainty of the concentration might be higher than stipulated (i.e. above
196 30 %), but the identity of the compound has been confirmed and was therefore considered appropriate to be
197 included in the subsequent data analysis.
198
199 *Data analysis*
200 All pesticide properties including effect concentrations (Tables S1 and S2) were acquired from the
201 Pesticide Properties Database (http://sitem.herts.ac.uk/aeru/ppdb/en/ accessed 18.08.2014) and from
202 the US EPA Ecotox Database (http://cfpub.epa.gov/ecotox/ accessed on 25.08.2014). In the cases
203 where more than one effect concentration was available for a pesticide, the lowest value was
204 selected. Legal status of the pesticides in Denmark and the EU was acquired from the Danish
205 Pesticide Database (http://middeldatabasen.dk/Middelvalg.asp accessed on 04.09.2014) and the EU
206 Pesticides Database (http://ec.europa.eu/sanco_pesticides/public/?event=homepage accessed on
207 04.09.2014), respectively (Tables S1 and S2).
208
209 For all water samples and sediment samples with pesticide detections above the LOD, the sum of
210 toxic units (SumTU) was calculated to standardise exposure concentrations according to a
211 benchmark organism. For water samples we used 96h growth inhibition tests on the green algae
212 *Pseudokirchneriella subcapitata* to benchmark sample toxicity to primary producers. In the cases
213 where no data existed, we used data for *Scenedesmus subspicatus* as an alternative. Acute 48h
214 mortality tests on *Daphnia magna* were used to benchmark the toxicity to invertebrates and 96h

mortality tests on *Oncorhynchus mykiss* were used to benchmark sample toxicity to fish. *Lepomis macrochirus* was used as an alternative species in the few cases where no data was available for *O. mykiss*.

The sum of toxic units (SumTU) is calculated as:

$$\mathrm{SumTU} = \sum_{i=1}^{n} \frac{C_i}{EC50_i} \quad (1)$$

where C_i is the concentration of pesticide *i* in the sample, and $EC50_i$ is the concentration of chemical *i* causing a 50% effect to the benchmark organisms.

Bed sediment and suspended sediment pesticide concentrations were converted to TU using 96h acute mortality tests for the sediment dwelling non-biting midge *Chironomus riparius* supplemented with 28d chronic exposure tests on emergence success for *C. riparius* in the cases where no 96h acute mortality test data existed. Often, only one of the tests was available for a pesticide, but in the few cases where data for both acute and chronic tests existed, we selected the lowest effect concentration. Effect concentrations in the *C. riparius* tests were based on measured pore water concentrations. In the cases where no sediment test data existed for a pesticide, we used the 48h LC50 for *D. magna* as surrogate measure for sediment toxicity. Plotting the *C. riparius* toxicity data as a function of 48h LC50 for *D. magna* for the pesticide compounds having both sets of toxicity data revealed that the deviation from the 1:1 line rarely exceeded one order of magnitude (Fig. S2).

Measured sediment-associated pesticide concentrations were converted to pore-water concentrations according to the equilibrium-partitioning approach to comply with the sediment benchmark toxicity tests that are based on dissolved phase pesticides in pore water. Moreover, pore

water concentrations are superior predictors of sediment toxicity to invertebrates compared to pesticides adsorbed to sediment particles (Xu et al. 2007).

Pore water concentrations from bed sediment and suspended sediment were calculated according to Ditoro et al. (1991) as:

$$C_{PW} = \frac{C_S}{K_d} \quad (2)$$

where K_d is the partitioning coefficient, C_S is the sediment concentration and C_{PW} the pore water concentration of the pesticide. K_d was calculated as:

$$K_d = K_{OC} \times f_{OC} \quad (3)$$

where K_{OC} is the dimensionless organic carbon-water partitioning coefficient for the pesticide and f_{OC} is the fraction of total organic carbon measured in the sediment sample. Kronvang et al. (2003) found the fraction of total organic carbon in bed sediments from 27 Danish agricultural streams to range from 5.5 to 16.1% with an average of 8.5%. Hence, the f_{OC} was set to 0.085 in our study. The K_{OC} was calculated as:

$$\log K_{OC} = a \times \log K_{OW} + b \quad (4)$$

where K_{OW} is the octanol-water partitioning coefficient. The constants a and b were set to 0.72 and 0.49, respectively, according to Schwarzenbach and Westall (1981).

We tested correlations between pesticide concentrations (ppm) among sample types (n = 19) using Spearman-Rank analysis. Stream specific (arithmetic) mean concentrations of storm flow samples were used. The number of storm flow samples ranged between two and five among streams (Table S4). Moreover, we tested correlations between sumTU of legacy pesticides and sum TU of contemporary pesticides within base flow, storm flow and sediment samples. For water samples, the correlations were based on data for all benchmark organisms. All data used in the Pearson

correlation analyses were log-transformed to obtain normal distribution. The Spearman Rank correation analyses were conducted in JMP 11.1.1 for Windows.

We tested if the addition of legacy compounds significantly increased the sumTU of water and sediment samples in control and agricultural streams, respectively, by comparing the sumTU of contemporary pesticides to the sumTU of all pesticides using Mann-Whitney tests in JMP 11.1.1 for Windows.

RESULTS AND DISCUSSION

Pesticide occurrence and toxicity patterns

We found a significant positive relationship among pesticide concentrations in all combinations of sample types ($P < 0.05$) (Table 1, Fig. S3). The strongest correlations were obtained between suspended sediment and bed sediment samples, between storm flow water and suspended sediment and between storm flow water and bed sediment (Table 1). Thus, streams with high pesticide concentrations in especially storm flow samples also had a high probability of having high pesticide concentrations in sediments and to a lesser extent during base flow. Importantly, SumTU based on contemporary pesticides was additionally a strong indicator for SumTU based on legacy pesticides in base flow samples (daphnia: $r = 0.724$, $P < 0.001$; fish: $r = 0.578$, $P = 0.009$; algae: $r = 0.460$, $P = 0.046$), storm flow samples (daphnia: $r = 0.603$, $P < 0.001$; fish: 0.468, $P < 0.001$; algae: $r = 0.359$, $P = 0.009$), suspended sediment samples (chironomids: $r = 0.563$, $P = 0.012$) and sediment samples (chironomids: $r = 0.696$, $P < 0.001$) (Fig. 1). This indicates that streams which are currently the most impacted by contemporary pesticide pollution, have probably also been so in the past. This is perhaps not surprising as areas with productive conventional agriculture rarely are converted into non-farming activities (Harding et al. 1998).

285

286 *Quantification of pesticide toxicity*

287 In 11 (≈ 17%), 12 (≈ 18%) and 35 (≈ 55%) of the storm water samples, pesticide concentrations
288 exceeded safety thresholds for daphnia (1/100 48h LC50), fish (1/100 96h LC50) and algae (1/10
289 96h EC50), respectively (Panel 2013) (Fig. 2, Table 2). Concentrations of legacy pesticides alone
290 exceeded the safety thresholds for daphnia and fish in six and three of the storm flow water
291 samples, respectively, while none of the samples contained legacy pesticide concentrations
292 exceeding the safety threshold for algae. Note however, that the average SumTU for daphnia, fish
293 and algae in agricultural streams all exceeded the respective safety thresholds (Table 2).
294 Importantly, and confirming the early findings of McKnight et al. (2015), the addition of
295 $SumTU_{D.magna}$ based on legacy pesticides to the $SumTU_{D.magna}$ based on contemporary pesticides
296 significantly increased the $SumTU_{D.magna}$ in storm water samples from agricultural streams (Fig. 2B,
297 P = 0.039). None of the base-flow water samples exceeded existing guideline values for
298 invertebrates, fish or algae (Fig. 2A, Table 2).

299

300 Sediment and suspended sediment samples contained pesticide concentrations exceeding safety
301 thresholds in 10 of 20 samples from agricultural streams. In seven of these samples, legacy
302 pesticide concentrations alone exceeded the safety threshold, and the addition of $SumTU_{C.riparius}$ for
303 legacy pesticides to the $SumTU_{C.riparius}$ for contemporary pesticides significantly ($\alpha = 0.1$) increased
304 the $SumTU_{C.riparius}$ in suspended sediments (Fig. 3, P = 0.038) as well as in bed sediments (Fig. 3, P
305 = 0.064). In fact, the average contribution of legacy pesticides to $SumTU_{C.riparius}$ for bed sediments
306 and suspended sediments was > 90%, and the average $SumTU_{C.riparius} > 0.1$ (Table 2).

307

Our results suggest that legacy pesticides can be highly significant contributors to the contemporary toxic exposure of stream biota, especially macroinvertebrate communities, and that those communities were primarily exposed to legacy pesticides via the sediment. However, Liess and von der Ohe (2005) and Schäfer et al. (2012) showed that stream dwelling macroinvertebrate communities were significantly different in streams containing peak flow concentrations of pesticides at 1/1000 48h $LC50_{D.magna}$, and this threshold was exceeded in approximately 50% of the storm water samples in our study (30% for legacy pesticides alone) (data not shown). This clearly suggests that the exposure of stream biota to dissolved phase legacy pesticides as well as legacy pesticides adsorbed to sediment particles are likely both important stressors in these streams. Integrating past land use should therefore improve the prediction of pesticide impacts on macroinvertebrate communities compared to the stringent focus on current use chemicals in the water and sediment phases (Harding et al. 1998). Highly important is the fact that our results, supported by the findings of McKnight et al. (2015), strongly suggest that disregarding legacy pesticides, in particular those adsorbed to sediment particles, in ecotoxicological field studies and pesticide monitoring programs probably leads to significant underestimations of total risk and significant underestimations of the relative importance of pesticides compared to other important anthropogenic stressors (Harding et al. 1998; Matson et al. 1997). However, we recognize that the bioavailability of the highly lipophilic pesticides adsorbed to particles may decrease with increasing age of the pesticide-particle complex (Xu et al. 2008). Hence the predicted sumTU for sediment-dwelling organisms may be overestimated when large proportions of the pesticide-particle complexes have been long-established.

Predicting the toxicity of pesticide mixtures based on the assumption of toxic additivity (Concentration Addition, CA), as done in the present study, may be problematic when the pesticides

in the sample have dissimilar Modes Of Action (MOA) (Belden et al. 2007; Cedergreen et al. 2013). However, CA appears to be a slightly conservative and broadly applicable model for pesticide mixtures with similar, dissimilar and unknown MOAs and has a relatively small risk of underestimating the effects (Backhaus and Faust 2012; Nowell et al. 2014). Moreover, the SumTU approach has been shown to strongly correlate with an ecological indicator for pesticide pollution (SPEAR) (Liess and von der Ohe 2005) and provides as strong a correlation to SPEAR as other models that consider different MOAs of sample constituents, e.g. the msPAF (Schäfer et al. 2013).

Potential sources of the legacy pesticides

The majority of the legacy pesticides included in this study (e.g. organochlorines and triazines) have the potential to persist for several decades in agricultural soils to which the compounds have been applied in the past (Aliyeva et al. 2013; Manz et al. 2001). In consequence, agricultural soils may still be important sources providing continuous fluxes of legacy pesticides to freshwater ecosystems (Barth et al. 2007; Gilliom 2007). The detection frequency of legacy pesticides was highest in base-flow water samples and sediment samples; although their concentrations increased 2 to 15 fold in water during storm flow (Table 2). This could indicate that a dominant source of legacy pesticides was upper soil layers in the catchments, originating from past agricultural applications, where surface runoff occurs (Manz et al. 2001). Re-suspension of contaminated sediment may have altered the partitioning between particle bound and dissolved phases of pesticides and hence could be an additional important source governing the observed increase in legacy pesticide concentrations during storm flow (Quesada et al. 2014). Additional sources of potential importance may include atmospheric deposition (Weber et al. 2010), point sources such as waste dumps (Aliyeva et al. 2013), industrial use and commercial products (Connor et al. 2007), and illegal private use (see

McKnight et al. (2015) for a detailed description of potential sources of legacy pesticides in streams).

Since the dominant source of legacy pesticides is likely agricultural soils, we expect the flux of legacy pesticides to streams to be relatively comparable between summer and winter, i.e. peaks associated with storm events in winter would be less strong than peaks associated with the additional application of contemporary pesticides in the summer. Data from the extensive Swedish pesticide monitoring program documents that legacy pesticides are still found in stream water outside the primary crop growing season of Nordic countries (Nanos et al., 2012). Hence, in contrast to contemporary pesticides, the toxic pressure of legacy pesticides in streams is likely relatively constant across seasons, additionally indicating that the relative toxic contribution of legacy pesticides to the sumTU increases outside the primary crop growing seasons.

Identifying compounds of concern

Among the legacy pesticides, the organophosphate chlorpyrifos and organochlorines such as DDT (and degradation products) and lindane were the strongest drivers of high SumTU for daphnia, fish and sediment dwelling invertebrates, whereas diuron and the triazine herbicides (terbutylazine and simazine) were the strongest drivers of high SumTU for algae (Table S5). Chlorpyrifos is still permitted for agricultural purposes in some EU countries but has been banned in Denmark since 2008. The remaining pesticides mentioned are forbidden for agricultural purposes in the EU (DDT since 1979, lindane since 2001 (but 1994 in Denmark), simazine since 2005, diuron since 2008 and terbutylazine since 2009).

Since the legacy pesticides significantly increased the $sumTU_{D.magna}$ in storm flow water and $sumTU_{C.riparius}$ in sediments we further evaluated the relative contribution of specific groups of pesticides to $sumTU_{D.magna}$ and $sumTU_{C.riparius}$ in storm flow water and sediment samples, respectively. The $sumTU_{D.magna}$ in storm flow water was most strongly influenced by contemporary pyrethroid insecticides (62.6%) and the legacy pesticide chlorpyrifos (15.3%) in agricultural streams, whereas the $sumTU_{D.magna}$ was most strongly influenced by legacy and contemporary pyrethroid insecticides (26.3% and 24.3%, respectively) and chlorpyrifos (42%) in control streams (Table 3). The $SumTU_{C.riparius}$ of suspended sediment and bed sediment samples were almost entirely governed by chlorpyirfos in agricultural streams whereas the $sumTU_{C.riparius}$, especially for bed sediments, was more influenced by organochlorine insecticides in control streams (Table 3). Since the half-life of chlorpyrifos in aquatic sediments is proposed to be 20-180 days (Mackay et al. 2014), which is comparable to the half-lives of pyrethroids, our findings could indicate that this active ingredient is illegally used in Denmark. Alternatively, as pointed out by McKnight et al. (2015), chlorpyrifos is well-known for its ability to undergo long-range transport and/or may still be permitted for use in material protection products (e.g. as a biocide).

Conclusions

Risk assessment, the identification of pesticides of particular concern and the prioritization of mitigation activities strongly rely on monitoring data from streams, and keeping up with the increasing number of (emerging) active ingredients entering the market remains a serious challenge. However, our results suggest that increasing attention should additionally be directed towards legacy pesticides due to their predicted high impacts on the biota of especially agricultural streams. Neglecting central legacy pesticides in stream monitoring programs may underestimate the predicted toxicity of stream sediments by up to 90%. Future assessment schemes and management

strategies should seek to quantify the actual toxicity of sediments containing high concentrations of legacy pesticides, and moreover seek to benchmark ecological entities of streams against more extensive pesticide screening programs, including legacy pesticides, in order to evaluate if the combined measurements of past and current use pesticides increase the explanatory power of correlations between all types of pesticides and their ecological effects. Monitoring programs should continuously re-address the status of legacy pesticides in freshwater systems to register developments in long term exposure profiles. To reduce costs, the frequency and concentration might be related to land-use history which can then be used as a proxy for potential exposure risk. Our understanding of pesticide exposure in streams needs expansion and should progress towards interpreting ecosystem responses in a temporal context where land use history is a key determinant to when and where to sample.

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SUPPORTING INFORMATION

Figures providing a schematic overview of the study sites, correlations between pesticide concentrations between sample types and relationships between compound-specific effect concentrations for *D. magna* and *C. riparius*. Tables presenting legal status and analytical detection limits for pesticides included in the field screening, stream and catchment characteristics, supporting statistical information and an overview of compounds responsible for highest sumTU.

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554 Fig. 1. SumTU for legacy pesticides as a function of the SumTU for contemporary pesticides for
555 base flow water samples (A), storm flow water samples (B) and sediment samples (C). Sediment
556 was sampled with two methods representing the bed sediment and suspended sediment. The
557 diagonal lines indicate 1:1 relationships. For all water samples, the SumTU was calculated for algae
558 (*R. subcapitata*), fish (*O. mykiss*) and invertebrates (*D. magna*), whereas SumTU calculations for
559 sediment samples were based on *C. riparius*.
560
561 Fig. 2. Average SumTU for base-flow water samples (A) and storm flow water samples (B).
562 SumTU is grouped according to stream category (control, n=9; agricultural, n=10) and according to
563 benchmark organisms (*D. magna*, *O. mykiss* and *R. subcapitata*). Asterisks indicate significant
564 differences in the pairwise tests at the 5% level (**). The boxplots display the median (bold line),
565 first and third quartiles (upper and lower end of box) and the 1.5-fold interquartile range (error
566 bars). Outliers are indicated with open circles.
567
568 Fig. 3. Average $SumTU_{C.riparius}$ for bed sediment and suspended sediment samples. SumTU is
569 grouped according to stream category (control, n=9; agricultural, n=10). Asterisks significant
570 differences at the 10% level (*) and 5% level (**).The boxplots display the median (bold line), first
571 and third quartiles (upper and lower end of box) and the 1.5-fold interquartile range (error bars).
572 Outliers are indicated with open circles.
573

574 Table 1. Results from the Spearman Rank analyses comparing the summed pesticide concentrations
575 (ppm) between all sample types. The correlation coefficients (r, first line) and significance levels (P,
576 second line) are given.

	Base-flow water	Storm flow water	Suspended sediment	Bed sediment
Base-flow water		0.658 0.002	0.523 0.026	0.694 < 0.001
Storm flow water			0.794 < 0.001	0.782 < 0.001
Suspended sediment				0.984 < 0.001
Bed sediment				

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578 Table 2. Overview of central parameters for the pesticides monitored during base-flow and storm
579 flow as well as in bed sediments (BS) and suspended sediments (SS). Parameter values are given ±
580 SE for control streams (n=9) and agricultural streams (n=10).

Parameter	**Control streams**	**Agricultural streams**
Base-flow water samples		
Average # compounds (all)	3.1 ± 0.9	8.8 ± 1.6
Average # compounds (legacy)	2.1 ± 0.4	4.1 ± 0.9
Average sum conc. (µg L^{-1}) (all)	0.033 ± 0.014	0.192 ± 0.099
Average sum conc. (µg L^{-1}) (legacy)	0.003 ± 0.001	0.055 ± 0.045
Average $SumTU_{D.magna}$ (all)	$6.8*10^{-6} \pm 3.9*10^{-6}$	0.0007 ± 0.0004
Average $SumTU_{D.magna}$ (legacy)	$1.3*10^{-7} \pm 1.7*10^{-8}$	0.0006 ± 0.0003
Average $SumTU_{O.mykiss}$ (all)	$1.7*10^{-5} \pm 8.7*10^{-6}$	0.0004 ± 0.0002
Average $SumTU_{O.mykiss}$ (legacy)	$6.3*10^{-7} \pm 6.8*10^{-9}$	$0.0002 \pm 6.3*10^{-5}$
Average $SumTU_{P.subcapitata}$ (all)	0.006 ± 0.003	0.036 ± 0.008
Average $SumTU_{P.subcapitata}$ (legacy)	0.0002 ± 0.00008	0.002 ± 0.002
Storm flow water samples		
Average # compounds (all)	7.7 ± 0.9	21.3 ± 1.4
Average # compounds (legacy)	3.5 ± 0.3	6.9 ± 0.5
Average sum conc. (µg L^{-1}) (all)	0.277 ± 0.088	1.845 ± 0.339
Average sum conc. (µg L^{-1}) (legacy)	0.045 ± 0.015	0.129 ± 0.018
Average $SumTU_{D.magna}$ (all)	0.002 ± 0.001	0.016 ± 0.007
Average $SumTU_{D.magna}$ (legacy)	0.001 ± 0.001	0.003 ± 0.001
Average $SumTU_{O.mykiss}$ (all)	0.004 ± 0.003	0.011 ± 0.003
Average $SumTU_{O.mykiss}$ (legacy)	0.001 ± 0.001	0.001 ± 0.001
Average $SumTU_{P.subcapitata}$ (all)	0.101 ± 0.045	0.892 ± 0.292
Average $SumTU_{P.subcapitata}$ (legacy)	0.004 ± 0.002	0.012 ± 0.005
Sediment samples		
Average # compounds (BS, all)	1.3 ± 0.5	5.9 ± 1.2
Average # compounds (SS, all)	2.1 ± 0.5	6.9 ± 1.1
Average # compounds (BS, legacy)	0.9 ± 0.4	3.8 ± 0.9
Average # compounds (SS, legacy)	1.3 ± 0.4	4.2 ± 0.8
Average sum conc. (µg kg^{-1} DW) (BS, all)	6.0 ± 2.5	65.1 ± 14.2
Average sum conc. (µg kg^{-1} DW) (SS, all)	13.1 ± 3.6	167.6 ± 57.0
Average sum conc. (µg kg^{-1} DW) (BS, legacy)	2.5 ± 1.1	22.7 ± 7.6
Average sum conc. (µg kg^{-1} DW) (SS, legacy)	6.6 ± 2.8	48.4 ± 21.3
Average $SumTU_{C.riparius}$ (BS, all)	0.0003 ± 0.0001	0.141 ± 0.083
Average $SumTU_{C.riparius}$ (SS, all)	0.001 ± 0.001	0.117 ± 0.090
Average $SumTU_{C.riparius}$ (BS, legacy)	$7.8*10^{-5} \pm 2.6*10^{-5}$	0.137 ± 0.082
Average $SumTU_{C.riparius}$ (SS, legacy)	0.001 ± 0.001	0.108 ± 0.090

581

582 Table 3. Relative contribution of selected groups of pesticides to the sumTU based on *D. magna* for
583 storm flow water samples and *C. riparius* for sediment samples. The values are grouped according
584 to the stream category (control and agriculture). The median sumTU values for the respective
585 samples are given.

		Storm flow water		Suspended sediment		Bed sediment	
		Control	Agriculture	Control	Agriculture	Control	Agriculture
	median sumTU	<0.001	0.004	<0.001	0.011	<0.001	0.014
Contemporary pesticides	Herbicide	5.9	6.9	70.4	0.9	22.8	2.3
	Fungicide	1.5	9.5	<0.1	<0.1	<0.1	<0.1
	Pyrethroid	24.3	62.6	<0.1	1.5	8.7	5.2
	Other insecticide	<0.1	0.9	<0.1	<0.1	<0.1	<0.1
Legacy pesticides	Herbicide	<0.1	<0.1	4.0	<0.1	<0.1	0.1
	Fungicide	<0.1	<0.1	25.6	<0.1	1.1	<0.1
	Organochlorine	NA	NA	<0.1	0.2	67.4	0.8
	Organophosphate	42.0	15.4	<0.1	97.4	<0.1	91.6
	Pyrethroid	26.3	2.7	<0.1	<0.1	<0.1	<0.1
	Other insecticide	<0.1	2.0	<0.1	<0.1	<0.1	<0.1

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